EFFECTS OF “BREED UP” ON NON-SEASONAL SPERMATOGENESIS IN OVINE

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ABSTRACT

Rams of Suffolk and Rambouillet influence were placed on a 60-d trial to determine the value of the trade product “Breed Up” in semen production through the summer months. Final collection was June 27 when ambient stress is generally at its highest point. Semen characteristics such as scrotal circumference, volume, concentration, and motility were used in the evaluation of the success of the product. While no significant values were recorded ($P < .05$), it was proven that the rams subject to the treatment group better maintained their ability to produce more fertile semen. Also, all rams within the treatment group showed an improvement in all four of the characteristics studied as opposed to those rams that were in the control group.

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INTRODUCTION

With an ever-increasing demand for food protein in our world's growing population, the need for small ruminant products to meet growing markets is becoming extremely important. The best way to initially maximize livestock production is to be able to be extremely reproductively efficient. Most ruminants, however, are typically short day breeders. Most ewes maximize reproductive efficiency when breeding occurs in the fall, followed by lambing in the spring. One of the problems that most producers face now is the demand for fall born lambs. The main complication with being able to raise fall born lambs comes with reproductive inefficiency in the ram. This is especially prevalent in the Suffolk or Hampshire rams, but across all breeds, over exposure to ambient temperature negatively affects fertility and conception rates during the late spring and early summer (Hulet et al. 1956). However, it is has been noted that any increase in nutritional quality will improve fertility in both the male and female. Due to an inability to be able to control environmental conditions or manipulate specific photo-periods in a commercial sheep setting, nutrition and stress limitations are really the only external factors that can be controlled to help aid in and increase in sperm quality. "Breed Up" is a new market product that has a specific nutritional break down that will meet the nutritional requirements necessary, and aid in testicular growth while also improving semen quality under high ambient stress. While science has proven that non-seasonal mating is typically due to the lack of infertility in the male, "Breed Up" will offer an alternative nutritional feed supplementation that allows producers to have a more successful lamb crop through spring and summer breeding.

OBJECTIVE

Determine how effective the trade product "Breed Up" is in manipulating semen characteristics in rams that are exposed to normal climactic heat stress. To evaluate the difference it has on breed type, as well as overall performance of the rams.

LITERATURE REVIEW

Livestock production in general is heavily affected by several external factors. All factors included play a role in productivity, as well as profitability. Some of these factors include dietary complications, reproductive failure, and even complications with growth and development (Terrestrial Animal Health, 2009).

Fertility issues are a concern in sheep production. Conditions imposed at semen collection time have much to do with these complications (Foote, 1978). The major contributor to variation in semen quality is the environment. Environmental effects may be temporary or permanent. Permanent effects occurring during pre-natal and pre-pubertal periods and temporary or permanent factors acting after spermatogenesis is initiated can alter semen quality. Semen quality improves during the first few months after puberty and declines in old age (Foote, 1978). In addition, the quality of ram spermatozoa changes markedly at different times of the year. The study of these seasonal trends is important to discover “normal” breed and individual variations (Green, 1940). In fact, McKenzie and Berliner (1937) demonstrated that seasonal variations in semen characteristics existed not only amongst rams but also between breeds. While each of these can be pinpointed as genetic flaws or complications, it is also true that they can be directly correlated to stresses brought on by environmental conditions. Despite both of these claims of inadequacy, the other outlying source that is a financial and productive burden upon industry is stress.

Stott (1981) defines stress in relation to animal husbandry as, “Stress denotes the magnitude of forces external to the bodily system which tend to displace that system from its resting or ground state.” Stott (1981) later goes on to say that stress interrupts the body’s ability to maintain complete physiological function at homeostasis. Stress is something that can be directly correlated with the success of any livestock operation. However, it is not a singular burden that producers must face or try to control; it can be generated from all different types of circumstances and ultimately allows for changes across homeostasis and well-being. He later alludes to the fact that stress can be either environmental, nutritional, or any number of things that brings the animal out of its normal resting state. According to Nyberg et al. (1988), stress creates a spontaneous acute reaction to the animal, and shows a high correlation to the amount of stress present to the concentration of plasma Cortisol present. This decreases the overall physiological performance and puts large costs on the entire industry. Dr. St-Pierre argues that, “2.4 billion dollars are lost annually due to stress less heat abatement” (St. Pierre et al. 2003). Stress is detrimental across all types of production settings. Morrison (1983) make the claims that stress causes all types of complications to ruminant productivity. He analyzed the effects that stress made on feed intake, heat transfer, significant reduction in growth rate, and the body’s ability to mobilize the immune system. These things combined attribute to the complications that arise from the simplest forms of stress.

This sheds light on one of the most detrimental stress related external factors of heat, and how drastically it not only affects the industry monetarily but also physiologically. Heat stress can be termed as the increase in air temperature, percent humidity, and the movement of air through space (Fuquay, 1981). Morrison (1983) attributes the majority of physiological complications to heat stress. Among other things he makes the argument that feed intake as well as the ability to sustain maintenance energy both decrease with the onset of heat stress. Heat stress is also directly correlated to inefficiency in reproductive physiology, and has provided producers with problems for years (Fuquay, 1981). Fuquay (1981) goes on to explain the complications that arise from animals trying to maintain thermoregulation when ambient temperature and humidity increase in their environment. He also explains the complications of heat transferred through the environment and the affects that it has on total performance across all species are detrimental to overall performance.

Ambient temperatures are more prevalent in some different geographical regions, but in west Texas it is something that producers constantly have to deal with. The ambient affect is not only detrimental directly to sperm production, but it is also a threat to several other factors such as appetite, temperature, and feeding patterns (Quiniou et al. 2000). These things combined all affect growth and development, and most importantly, play a huge role in maturation and reproductive efficiency. The ambient affect, as stated, can alter maturation of spermatozoa, but also the external symptoms can corrupt physiological homeostasis. While controlling environmental conditions through excellent facilities is the best way to control the ambient affect, it’s rarely the most cost effective and almost never an option for producers in the southwest part of the United States. The only option for most producers to controlling ambient stress is to try and aid in maintenance and improvement through diet and nutrition. While many experiments in the past have come with little to no avail, there is still wide belief it is the best way to maximize efficiency during these times of the year.

Extremely high ambient temperatures can affect both male and female physiology and can cause extreme difficulties when out of season breeding is desired. The onset of heat stress causes a direct release of Adrenocorticotropic Hormone (ACTH) which controls the release of Cortisol (Hansen and Arechiga, 1997). High Cortisol levels are directly correlated with a negative effect on reproduction of farm animals, but more importantly it alters gonadal function (Wise et al., 2000). Suriyasomboon et al. (2005) conducted and experiment with 110 sexually mature boars, and tested semen response against different housing protocols. They concluded an extreme seasonal affect, from a specific two-month period, on percent normal spermatozoa, percent abnormalities (morphology) and motility. This period was pinpointed as the summer months, and it affected all boars across all different housing methods. The final conclusion was that heat and humidity had a large negative affect on the semen quality of all boars across every setting. At the same time, according to the Green (1940), study the presence of minute sperm seems to be associated with summer months. Very few were noticeable during May. However, in June they were present at the rate of 23/500 cells. This number reached 46/500 in July and later diminished to 2/500 in October. These forms have been only rarely observed during the winter and early spring months. The mean maximal temperatures at Minneapolis during the Green (1940) study were as follows: January,27; February,20; March,39; April,52; May,76;

June,78; July,85; August, 82; September, 75; October, 56; November, 48; December, 36. Green (1940) also stated that in summary, the quality of the semen decreased slightly but steadily from January to May. During June and July the ejaculates became quite inferior presenting an increased number of head abnormalities, tailless heads, and minute sperm. From July to October, a very rapid readjustment toward higher quality was under way. After October all of the sperm types remained relatively stationary in their proportional relationships. The density likewise started to decline in May, reaching its lowest level in August. The rate of recovery lagged about one month behind change in normal cells, the maximum density not being attained until December. This reinforces that it is extremely difficult for all species to breed outside of their normal mating patterns. This includes trying to breed out of season, and sometimes in a season that's extremely irregular in its climate pattern.

Ovine, more specifically, are short day breeders and tend to come into their natural breeding cycle during the fall. In fact, changes in profiles of serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and in mating performance and ejaculate volume have been determined during the ovine breeding season (Sanford et. al, 1977). Sanford et al., (1977) also determined that there were changes in FSH and testosterone in rams throughout the fall breeding seasons and differences among breeds. Although it is breed-specific, ovine typically do not have high conception rates out of their natural season. There have been attempts to try and manipulate seasonality through exposure to light period, and some progress has been made in increasing non-seasonal conception rates. However, Sanford et al.

(1977) continues this argument and concluded that seasonal variations in the pattern of LH release in the ram are thought to reflect alterations in the hypothalamic-hypophyseal function, brought about primarily by changes in photoperiod. In 2010, Cameron et al. conducted an experiment to evaluate the reproductive performance of ewes and rams exposed to four month protocols of long days (LD) and short days (SHD). Long days were considered 16 hours of light, and short days were considered to only be exposed to 8 hours of light. This project was designed to test the response of estrus and conception rates by manipulating the photoperiod in an accelerated lambing program. Ewes were exposed to 4 month periods of LD, and then an aggressive 50 day period of SHD. Thirty-five percent of ewes were cycling at the completion of SHD, even though it was out of the natural mating season, and eighty percent were showing signs of luteal activity with the introduction of the ram. This revealed that there was a response to manipulation of the photoperiod, and even though the rams were subject to the same treatment they performed similarly to the ewes (J. Cameron et al. 2010). Similarly in the ram, photo-period subjection would also increase fertility through melatonin secretion. In a project conducted by Fitzgerald and Stellflug (1991) rams were subject to either 16 weeks long worth of long days (16L:8D) or short days (8L:16D), and then the treatment groups were injected with melatonin for 40-80 days at the peak of scrotal circumference. Two of the treatment groups were injected before scrotal regression had occurred, and one was not started until after scrotal regression had started. Scrotal regression begins to occur when rams come out of their natural fall breeding pattern and enter periods of longer exposure to sunlight. The test proved that rams that had received melatonin injections has a higher percent melatonin present in the blood, and also testicular growth was recorded in rams treated with melatonin. The other comparative analysis indicated that test groups I and III percent fertility in the spring (86%-91%) was equal to that of control groups in the fall (93%). Also, fertility was greater (P less than .05) when compared to control groups in April (59% to 62%), and finally, net lamb production was greater in ewes that were bred in the spring in rams that were treated with melatonin or exposed photoperiods than that of the non-treated control group (Fitzgerald and Stellflug. 1991). It is evident the effects of daylight on the secretion melatonin not only stimulate estrous in the female, but also it has testicular implications and increases fertility in the male.

However, complications are still present with trying to increase these conception rates through production settings when manipulating photoperiods simply are not an option for the majority of producers. One of the main complications for sheep producers is the fertility of the ram during the summer months. When rams are exposed to high ambient temperatures, there is a negative effect on motility and percent live sperm (Brooks and Ross. 1962). This is true with all species. However, when the testes are exposed to a consistent level of highly elevated temperatures sperm are destroyed and the fertility of the animal is minimal. Also, since it takes approximately 56 days for semen maturation and transport, it is easy to note that one single ambient exposure to heat can ruin almost an entire breeding season (Foote, 1978). There are three main organs that are associated with reproduction in the ram: the pituitary, testis, and thyroid (Berliner and Warbritton, 1937). Berliner and Warbritton (1937) explain how the testes are directly affected by heat and cold stress, and that activity of the thyroid is correlated with the amount of semen production in rams. Their experiment helped to prove that the ambient affect on particular tissues coincide with the release of thyro-tropic hormone altered semen production and was very seasonally-specific. An experiment conducted by Brooks and Ross reinforces the fact that, specifically in rams, percent motility, live sperm, and concentration were more heavily affected than concentration and volume (Brooks and Ross. 1962). This experiment was unique in that it extended knowledge of the actual effects of heat on fertility and not just exposure to light. Gun et al. (1942) make the initial claim that light was absolutely detrimental when exposed to semen and kills all live sperm cells. Brooks and Ross (1962), however, designed a controlled experiment that exposed elevated ambient temperatures, within a confinement setting, and then tracked their performance and data. In a similar experiment performed in 1957 by Dutt and Simpson, they concluded that not only was fertility lower in rams exposed to high summer temperatures, but also percentage embryonic-mortality was much higher as well. As a result of this they concluded that at lower motility rates it could be assumed to have a higher embryonic mortality rate (Dutt and Simpson, 1957). This experiment was crucial in understanding that heat alone, when tested singularly, was absolutely detrimental to the physiological process of production and maturation of sperm cells.

It is important to consider that while it is assumed that heat tolerance and stress affect the species as whole, the reaction and ability to maintain a more consistent level of homeostasis is very breed specific (Scharf et al. 2010). Scharf et al. (2010) cite a very classic comparison of Bos Taurus versus Bos Indicus cattle, and the ability of the Bos Indicus cattle to better withstand greater levels of heat stress. Berliner and Warbritton (1937) examined the difference in semen performance of two different types of ram breeds. The experiment studied Hampshire and Shropshire rams and their sperm production across different seasons, as well as the activity of their thyroid. The experiment concluded that not only did thyroid activity help regulate sperm production, but that naturally Hampshire rams had a greater affinity to higher levels of sperm production in a-typical breeding season months than did that of Shropshire rams. This study helps to reestablish the fact that genetic disposition allows for some animals to better cope with ambient conditions.

On the other hand, it is known that heritability of fertility is extremely low, (Rollinson, 1955) other factors have to be considered when manipulating the influence on reproductive efficiency. Factors such as nutrition, environment, and stress all play as vital of a role in fertility as the animals' genetic composition. While a controlled environment has proven to be successful in improving fertility in rams; the ability to provide this kind of assistance across production agriculture is simply not economically feasible. The industry has to pursue different ideas through nutritional supplementation and stress limiting protocols to help aid in improving fertility through these detrimental months (Fuquay, 1981). Nutrition probably offers producers the greatest form of impact that they can directly have across all livestock. It is readily known and recorded that supplementation or flushing, rapidly improves conception rates of females (West et al.1991). It is equally important, however, to supplement the male species prior to breeding. Through supplementation producers can limit nutritional stress, which also aids in improvement of dietary synthesis of all necessary nutrients. Mader and Davis (2004) make the claim that altering the feeding regimen can limit the effect heat stress and can have on the animal. Their experiment revealed that feeding animals later in the day improved Dry Matter Intake (DMI), as opposed to the morning or middle of the day. Also, another experiment they conducted showed that by limiting energy in the diet, and substituting with dietary roughage decreased the incidence of cattle being susceptible to heat stress due to less heat created from lower metabolic energy levels (Mader and Davis, 2004).

Terriil et al. (1948) claim that environmental conditions had the greatest affect on body weight and condition score of all the traits studied in their test of Rambouillet rams. Their study was designed to test the affects of environmental conditions of several different types of fleeces traits as well as body weight and condition scores. This experiment gives a lead into the importance of maintain body weight and body condition scores (BCS) through adverse or ambient climactic conditions. Another measure of fertility as explained by Martinez-Velasquez (2003) that Scrotal Circumference (SC) is a direct indicator of greater reproductive performance. Similarly, the process of flushing as described by Torell et al. (1972) shows the added benefits to increasing the plane of nutrition to ewes prior to the breeding season. They discovered substantial statistical increase in lambing percentages, and determined that the added reproductive efficiency of the increased lamb crop far outweighed the initial inputs in terms of feed costs.

Research has proven that simple supplementation and increases in feed intake alone do not directly correlate to improvements in volume or concentration of semen (Stevermer et al. 1961). However, supplementation of vitamins and minerals has shown some improvements in semen quality. In a study conducted by Audet et al., (2004) three separate trial groups of 12 month old boars were subjected to four treatment groups supplementing different levels of vitamins. The four groups included: supplementing basal diet including vitamins and minerals, basal diet of vitamins and minerals with vitamin C added, basal diets with fat soluble vitamins, and a basal diet with water soluble vitamins. The results indicated that semen collection from boars that had been supplemented basal diets with water soluble vitamins added had a greater level of semen production, and a drastic increase in sperm motility. Evaluation of semen revealed higher levels of folic acid concentration in the water-soluble treatment group which aided in the improvement (Audet et al. 2004). Dutt and Barnhart (1959) examine the importance of supplementing total plain of nutrition in terms of percent and Total Digestible Nutrients (TDN). Their study revealed that not only did a higher plane of nutrition increase volume of semen, but also the onset of puberty was at an earlier in age in those animals that received 100% TDN from weaning to sexual maturity. These studies combined prove that nutrition plays a vital role in the development and maturation of semen and effective sperm cells, and the complications with seasonality can be met with different types of production influences.

MATERIALS AND METHODS

Thirty mature Rambouillet and Suffolk rams were randomly selected for a feeding trial that began on February 6^{th}, 2012 at the Angelo State Management Instruction and Research (MIR) Center in San Angelo, Texas. All rams were brought in off a pasture setting in Miles, TX., and started on a full feed pre-conditioning phase in a pen setting for approximately 60 days. The rams were divided into 4 separate pens to allow for adequate room and closer inspection of health and wellness. The rams were fed a ration of six pounds a day and supplemented with alfalfa hay three times a week and had clean fresh water and shade available ad libitum. At the completion of the pre-condition phase, all rams were individually stimulated via electro-ejaculation, collected, and evaluated. Volume, concentration, and a motility score were given, and Scrotal Circumference was measured and recorded. Breed type was also recorded and used to evaluate data.

At the completion of the pre-conditioning phase and initial semen collection, the rams were randomly assigned to either a control or treatment group; where the rams were put on separate feed rations. Eleven rams were put into the control group, while 19 rams were placed into the treatment group and were started on the "Breed Up" ration. They were fed six pounds a day per animal, and provided alfalfa hay three times a week ad libitum. The trial phase lasted sixty days and the rams were then collected through electro-ejaculation, and semen measurements were recorded.

Volume, concentration, motility score, and scrotal circumference were analyzed using the GLM procedures of SAS (Cary, NC) with each individual animal being identified separately and compared across treatment and breed type. Treatments will be considered different when alpha is 0.05 or less.

RESULTS AND DISCUSSION

Breed influences played a role in initial differences as well as change in scrotal circumference (SC), concentration (Con.), volume (Vol.), and motility (Mot.) The Suffolk rams had a smaller SC ($P < .05$), but were similar in terms of Con., Vol., and Mot. ($P > .05$). Similarly when comparing initial values from control to the treatment group the values from the initial collection were similarly correlated ($P > .05$).

Scrotal Circumference

The comparison of initial SC to final SC in Table 1 shows the increase in cm. of the increase in SC of the treatment group. While it is not significant ($P > .05$), it is important to note the difference from initial to final and the added performance of the "Breed Up" group. The change in the "Breed Up" group ($0.81 \times 10^{9)}$ shows the added performance from the higher plane of nutrition that the supplement has to offer. Martinez-Velasquez (2003) state through their experiment that scrotal circumference is directly related to fertility, and even though Table 1 does not represent a significant P-Value ($P < .05$) it is still valuable to show the increase in size (cm.) and ultimately fertility.

Volume

Table 2 specifically analyzes the volume of semen in mL. and the results show that the rams from the control group has a much greater initial volume (3.66 x

Table 1. Initial, Final, and Change in Scrotal Circumference of both treatment and control groups of ram's being tested for "Breed Up."

	Treatment			
	Control	Breed Up	Standard Error	P-Value
Scrotal Circumference, cm. (Initial)	33.09 ± .01	32.57 ± .01	0.92 ± .01	0.6636
Scrotal Circumference, cm. (Final)	33.40 ± .01	33.39 ± .01	0.98 ± .01	0.9909
Scrotal Circumference, cm. (Change in SC)	0.31 ± .01	0.81 ± .01	0.31 ± .01	0.2130

Table 2. Initial, Final, and Change in Volume of both treatment and control groups of ram's being tested for "Breed Up."

	Treatment			
	Control	Breed Up	Standard Error	P-Value
Initial Volume, mL.	3.66 ± .01	2.92 ± .01	0.31 ± .01	0.0709
Final Volume, mL.	3.06 ± .01	3.24 ± .01	0.33 ± .01	0.6636
Change in Volume, mL.	-0.60 ± .01	0.32 ± .01	0.46 ± .01	0.1272

10^9 mL) than those of the treatment group (2.92×10^9 mL). Fuquay (1981) shows the effects of heat stress and fertility and the relevance of that study to this project is evident in the fact that not only did the "Breed Up" group have an increase in semen volume, but the fact that the final collection was made in the middle of the summer months (June 27th) had an adverse affect and decreased actual average volume from the control group. While understanding the ambient affect that Fuquay (1981) explains in his experimental procedures, it is evident that the heat stress brought about by the environment played a negative effect on the control group. Alternatively, even though there was no significance in the final volume ($P > .05$) the ability of the "Breed Up" group to increase the change in the volume on average .32 mL, and that it maintained and did not show the negative effects of ambient temperature like the control group.

Concentration

Initial concentration levels between the treatment and control groups were considered to be similar ($P > .05$). By analyzing the final concentration and the change in concentration from Table 3 the results reveal a statistical increase in concentration for the "Breed Up" group. While the data is not considered significant ($P > .05$), it is similar, like volume, that the treatment group did not suffer from the ambient effect of heat stress like the control group. The control group shows a negative relation with ambient effect due to the decrease in final concentration on the June 27th collection date. Again, these numbers do not match up to be

Table 3. Initial, Final, and Change in Concentration of both treatment and control groups of ram's being tested for "Breed Up."

	Treatment			
	Control	Breed Up	Standard Error	P-Value
Initial Concentration	2.50×10^9	1.94×10^9	2.78×10^8	0.1195
Final Concentration	2.37×10^9	2.17×10^9	2.68×10^8	0.5573
Change in Concentration	-1.28×10^9	2.32×10^9	4.34×10^8	0.5135

Table 4. Initial, Final, and Change in Motility of both treatment and control groups of ram's being tested for "Breed Up."

	Treatment			
	Control	Breed Up	Standard Error	P-Value
Initial Motility	4.45×10^9	4.05×10^9	0.37±.01	0.4029
Final Motility	4.18×10^9	4.36×10^9	0.30±.01	0.6265
Change in Motility	-0.27×10^9	0.31×10^9	0.48±.01	0.3419

significantly representative because of its P-Value, yet it still shows the treatment groups ability to better maintain reproductive function through adverse summer conditions than those rams delegated to normal forms of supplementation without “Breed Up.

Motility

Similar to volume and concentration, motility at the original collection date was similar in both the control and treatment groups ($P > .05$). Goerke et al. (1970) makes the statement in his research that a higher percent motility relates to a greater correlation of percent normal sperm in Southdown rams, which would correlate to higher conception rates to ewes’ exposed. There was no statistical significant difference at the final ejaculation ($P > .05$), but the fact that there was an increase in motility score of the “Breed Up” group versus the decline of the control group indicates some substantial effects to the supplement. Though not significantly represented through the data, the added effects of the treatment through the non-seasonal ambient conditions allows for maintained or increased fertility versus conventional supplementation.

IMPLICATIONS

The rams used within this performance, though not showing statistical significant changes in semen quality, did trend to be affected by the supplementation of “Breed Up.” While considering the ambient affect that limits high performing semen production, the rams treated with “Breed Up” not only maintained consistent levels through the middle of the summer months, but also improved slightly as compared to those subject to the control. All four measurements of semen quality showed an increase from the initial to final collection, and because breeds were allocated randomly to each treatment group effects were consistent across the board. Though it is necessary for increased research on the product, the positive trend and correlation to “Breed Up” rams shows that there may be benefits to supplementing this product when there is a need for non-seasonal, ovine reproductive services.

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